

CLAIMS

We claim:

1. A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

- 5 a) fixing and permeabilizing said cells;
- b) catalyzing the deposition of tyramide in cells comprising said intracellular analyte;
- c) contacting said cells with a detectable label that directly or indirectly binds to tyramide, whereby cells comprising said intracellular analyte are specifically labeled; 10 and
- d) detecting a signal from cells comprising said detectable label using a flow cytometric device, whereby said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.

2. A method of detecting the presence of an intracellular analyte in one or more cells 15 by flow cytometry, the method comprising:

- a) fixing and permeabilizing said cells;
- b) catalyzing the deposition of tyramide conjugated to a detectable label in cells comprising said intracellular analyte, whereby cells comprising said intracellular analyte are specifically labeled; and
- 20 c) detecting a signal from cells comprising said detectable label using a flow cytometric device, whereby said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.

3. A method according to claim 1 or 2, wherein said signal is at least 20-fold greater than a signal obtainable by standard flow cytometry methods.

25 4. A method according to claim 1 or 2, wherein said signal is at least 50-fold greater than a signal obtainable by standard flow cytometry methods.

5. A method according to claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a binding partner that specifically binds to said analyte, wherein said binding partner is conjugated to an enzyme capable of catalyzing the deposition of tyramide;

(ii) removing unbound binding partner from said cells; and

5 (iii) contacting bound binding partner with tyramide, whereby said enzyme catalyzes the deposition of tyramide in cells comprising said intracellular analyte.

6. A method according to claim 5, wherein said binding partner is incubated with said fixed and permeabilized cells in a medium comprising at least about 50% serum.

7. A method according to claim 6, wherein said serum is fetal bovine serum.

10 8. A method according to claim 7, wherein said medium comprises at least about 95% fetal bovine serum.

9. A method according to claim 8, wherein said medium further comprises about 0.2% saponin.

15 10. A method according to claim 1 or 2, wherein said cells are permeabilized in a medium comprising saponin.

11. A method according to claim 1 or 2, wherein said cells are permeabilized in a medium comprising methanol.

12. A method according to claim 5, wherein said bound binding partner is contacted with tyramide in a medium comprising an aprotic solvent.

20 13. The medium of claim 12, wherein said medium comprises at least about 5% of an aprotic solvent selected from the group consisting of acetone, dimethyl sulfoxide, acetonitrile, and dimethyl formamide.

14. A method according to claim 1 or 2, wherein said detectable label is a fluorochrome.

15. A method according to claim 14, wherein said fluorochrome comprises a fluorescent molecule selected from the group consisting of fluorescein, phycoerythrin, CY5, allophycocyanine, Texas Red, peridinin chlorophyll, and cyanine.
16. A method according to claim 5, wherein said enzyme is selected from the group consisting of hydrolysases, peroxidases, oxidases, esterases, glycosidases and phosphatases.
17. A method according to claim 5 wherein said enzyme is horseradish peroxidase.
18. A method according to claim 1 or 2, wherein said catalyzing step comprises:
- 10 (i) incubating the fixed and permeabilized cells with a first binding partner that specifically binds to said analyte, and a second binding partner that specifically binds to said first binding partner, wherein said second binding partner comprises an enzyme, wherein said second binding partner is conjugated to an enzyme capable of catalyzing the deposition of tyramide;
- 15 (ii) removing unbound second binding partner from said cells; and
- 15 (iii) contacting bound second binding partner with tyramide, whereby said enzyme catalyzes the deposition of tyramide in cells comprising said intracellular analyte.
19. A method according to claim 18, wherein said second binding partner is an immunoglobulin-enzyme conjugate.
20. A method according to claim 19, wherein said second binding partner is incubated with said fixed and permeabilized cells in a medium comprising at least about 50% serum.
21. A method according to claim 20, wherein said serum is fetal bovine serum.
22. A method according to claim 21, wherein said medium comprises at least about 95% fetal bovine serum.
25. A method according to claim 19, wherein said immunoglobulin-enzyme conjugate is selected from the group consisting of immunoglobulin-peroxidase, immunoglobulin-

hydrolase, immunoglobulin-oxidase, immunoglobulin-glycosidase and immunoglobulin-phosphatase.

24. A method according to claim 23, wherein said immunoglobulin-enzyme conjugate is immunoglobulin-horseradish peroxidase.

5 25. A method according to claim 1 or 2, wherein said one or more cells are one or more mammalian cells.

26. A method according to claim 25, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets, lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells, Jurkat cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres, endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells, Langhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.

10 27. A method according to claim 1 or 2, wherein said one or more cells are cultured cells.

15 28. A method according to claim 1 or 2, wherein said intracellular analyte is selected from the group consisting of intracellular cytokines, antigens, viral antigens, nuclear antigens, cytoplasmic antigens, organellar antigens, enzymes, cytoskeletal molecules, glycolipids, lipids, glycans, chaperones, RNA, DNA, messenger RNA, ribosomal RNA, signal transduction proteins, and structural proteins.

20 29. A method according to claim 1 or 2, wherein said said intracellular analyte is not a natural component of said one or more cells.

30. A method according to claim 1 or 2, wherein said intracellular analyte cannot be detected by standard flow cytometry methods.

25 31. A method according to claim 1 or 2, wherein said one or more cells are obtained from a patient.

32. A method according to claim 31, wherein said signal is correlated to a diagnosis of a disease in said patient.

33. A kit for performing a method according to claims 1 or 2.

34. A composition for use in an assay to detect an analyte of interest, comprising:

5 a binding partner that specifically binds to said analyte; and

a medium comprising at least about 50% serum, wherein said binding partner is not a natural constituent of said serum.

35. A composition according to claim 34, wherein said serum is fetal bovine serum.

36. A composition according to claim 35, wherein said medium comprises at least 10 about 95% fetal bovine serum.

37. A composition according to claim 36, wherein said medium further comprises about 0.2% saponin.

38. A composition for use in an assay to detect an analyte of interest, comprising:

15 tyramide conjugated to a detectable label;

an enzyme capable of catalyzing the deposition of tyramide; and

a medium comprising at least about 5% by volume of an aprotic solvent.

39. A composition according to claim 38, wherein the aprotic solvent is selected from the group consisting of acetone, dimethyl sulfoxide, acetonitrile, and dimethyl formamide.

40. A composition for use in an assay to detect an analyte of interest, comprising:

20 tyramide conjugated to a detectable label;

an enzyme capable of catalyzing the deposition of tyramide; and

a medium having an ionic strength of about 0.1 M or less.

41. A composition according to claim 40, wherein said medium is selected from the group consisting of water and borate buffer.

42. A composition according to claim 40, wherein said medium has a pH of between about 7 and about 9.

43. A composition according to claim 40, wherein the medium comprises an aprotic solvent

5 44. A composition according to claim 43, wherein the aprotic solvent is selected from the group consisting of acetone, dimethyl sulfoxide, acetonitrile, and dimethyl formamide.

45. A composition according to claim 43, wherein the medium comprises at least about 5% by volume of said aprotic solvent.

46. A composition for use in an assay to detect an analyte of interest, comprising:

10 tyramide conjugated to a detectable label;

an enzyme capable of catalyzing the deposition of tyramide; and

a medium comprising one or more amino acids, dipeptides, and/or oligopeptides.

47. A composition according to claim 46, wherein said medium has a pH of between about 7 and about 9.

15 48. A composition according to claim 47, wherein the medium comprises an aprotic solvent

49. A composition according to claim 48, wherein the aprotic solvent is selected from the group consisting of acetone, dimethyl sulfoxide, acetonitrile, and dimethyl formamide.

50. A composition according to claim 48, wherein the medium comprises at least about 20 5% by volume of said aprotic solvent.

51. A composition according to claim 46, wherein said medium comprises about 20 mM glycylglycine, and about 1 M NaCl, and is at a pH of about 8.

52. A composition for use in an assay to detect an analyte of interest, comprising:

25 a hapten-conjugated first antibody or antibody fragment, that specifically binds

said analyte of interest;

an enzyme-conjugated second antibody or antibody fragment, that specifically binds said hapten-conjugated first antibody; and

a precipitate formed by the enzymatic activity of said enzyme conjugated to said second antibody.

- 5 53. A composition according to claim 52, wherein said hapten is selected from the group consisting of fluorescein isothiocyanate, dinitrophenol, and trinitrophenol.
54. A composition according to claim 52, wherein said enzyme is selected from the group consisting of hydrolysases, peroxidases, oxidases, esterases, glycosidases and phosphatases.
- 10 55. A composition according to claim 54 wherein said enzyme is horseradish peroxidase.
56. A composition according to claim 52, wherein said precipitate comprises tyramide.
- 15 57. A composition according to claim 52, further comprising one or more cells comprising said analyte of interest, wherein said one or more cells are not attached to a surface.
58. A composition for use in an assay to detect an analyte of interest, comprising:
an enzyme-conjugated antibody or antibody fragment, that specifically binds said analyte of interest; and
a precipitate comprising tyramide formed by the enzymatic activity of said enzyme conjugated to said second antibody.
- 20 59. A composition according to claim 58, wherein said enzyme is selected from the group consisting of hydrolysases, peroxidases, oxidases, esterases, glycosidases and phosphatases.
60. A method according to claim 59 wherein said enzyme is horseradish peroxidase.

61. A composition according to claim 58, further comprising one or more cells comprising said analyte of interest, wherein said one or more cells are not attached to a surface.